

Vessels in Roots and Rhizomes of *Dryopteris crassirhizoma* (Dryopteridaceae) from Heilongjiang Province, China

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ABSTRACT.—In the present study, tracheary elements in roots and rhizomes of *Dryopteris crassirhizoma* were observed with scanning electron microscopy (SEM). SEM observation revealed that all tracheary elements in both organs were vessels. These vessel elements have end-wall perforation plates and lateral-wall perforation plates. End-wall perforation plates in roots are more specialized than in rhizomes; they are all scalariform and obliquely positioned in end walls. Most end-wall perforations, especially in the center portion of end-wall perforation plates, lack pit membrane remnants, but pit membrane remnants are relatively abundant in some end-wall perforations of roots and rhizomes. It is noteworthy that several larger perforations on lateral walls usually are grouped together and form local lateral-wall perforation plates. Wide perforations alternating with narrow perforations characterize vessels of roots and rhizomes. In addition, the majority of perforations in lateral-walls have porose pit membranes or pit membrane remnants, range from intact pit membrane to nearly devoid of pit membrane remnants. Some vessels in rhizomes have several facets in which long scalariform pits have various degrees of porose membranes. These vessels contact several other tracheary elements for transferring materials.

In his classic study on fern tracheary elements, White (1961,1963) not only reported that vessels occur in *Pteridium* and *Marsilea*, but also used such terms “presumptive vessel” or “tracheary elements with end plates” to describe vessel-like tracheary elements in some ferns, such as *Dryopteris thelypteris* and *Woodsia obtusa*. The end plates of those cells are highly specialized in comparison with the lateral walls; scalariform pits on the end walls are wider than these on the lateral walls. Because of limitations of light microscopy, White (1963) was unable to determine the degree of pit membranes on end plates of those vessel-like tracheary elements (i.e. whether entire pit membranes on end plate were present or absent). The nature and presence of vessels are not revealed with light microscopy. Recently, Carlquist and Schneider (1997a,b) have confirmed by means of SEM that vessel-like tracheary elements as described by White (1963) in *Astrolepis* and *Woodsia* are vessels. SEM observations on those ferns showed that so-called pits on end walls are actually perforations with various degrees of pit membrane remnants from minutely to clearly absent, and revealed that there are lateral-wall perforations. It was noted that those genera, which grow in seasonally dry and cold places, have varying differentiated vessel elements in their roots and rhizomes in these authors’ studies (Carlquist and Schneider 1997a,b, 1998a,b, 1999; Carlquist, Schneider and Yatskievych, 1997; Schneider and Carlquist 1997, 1998a,b, c, 1999). These studies seem to further support the hypothesis that fluctuation

in water availability appears basic to evolution of vessels in vascular plants (Carlquist 1975). Ferns distributed in Heilongjiang province can be formally divided into four different ecological groups according to plant-water relationships as follows (Ao and Li, 1987):

1. Xerophytes: *Selaginella sibirica*, *S.tamariscina*, *Aleuritopteris argentea*, *Dryopteris fragrans*, *Lepisorus ussuriensis* and *Pyrrosia petiolosa* etc.
2. Mesophytes: *Adiantum pedatum*, *Athyrium multidentatum*, *A.yokoscense*, *Dryopteris crassirhizoma*, *Equisetum pratense* and *E.silvaticum* etc.
3. Helophytes: *Matteuccia struthiopteris*, *Osmunda cinnamomea* var.*asiatica*, *Onoclea sensibilis* and *Thelypteris palustris* etc.
4. Hydrophyte: *Equisetum fluviatila*, *Marsilea quadrifolia*, *Azolla filicoides* and *Salvinia natans* etc.

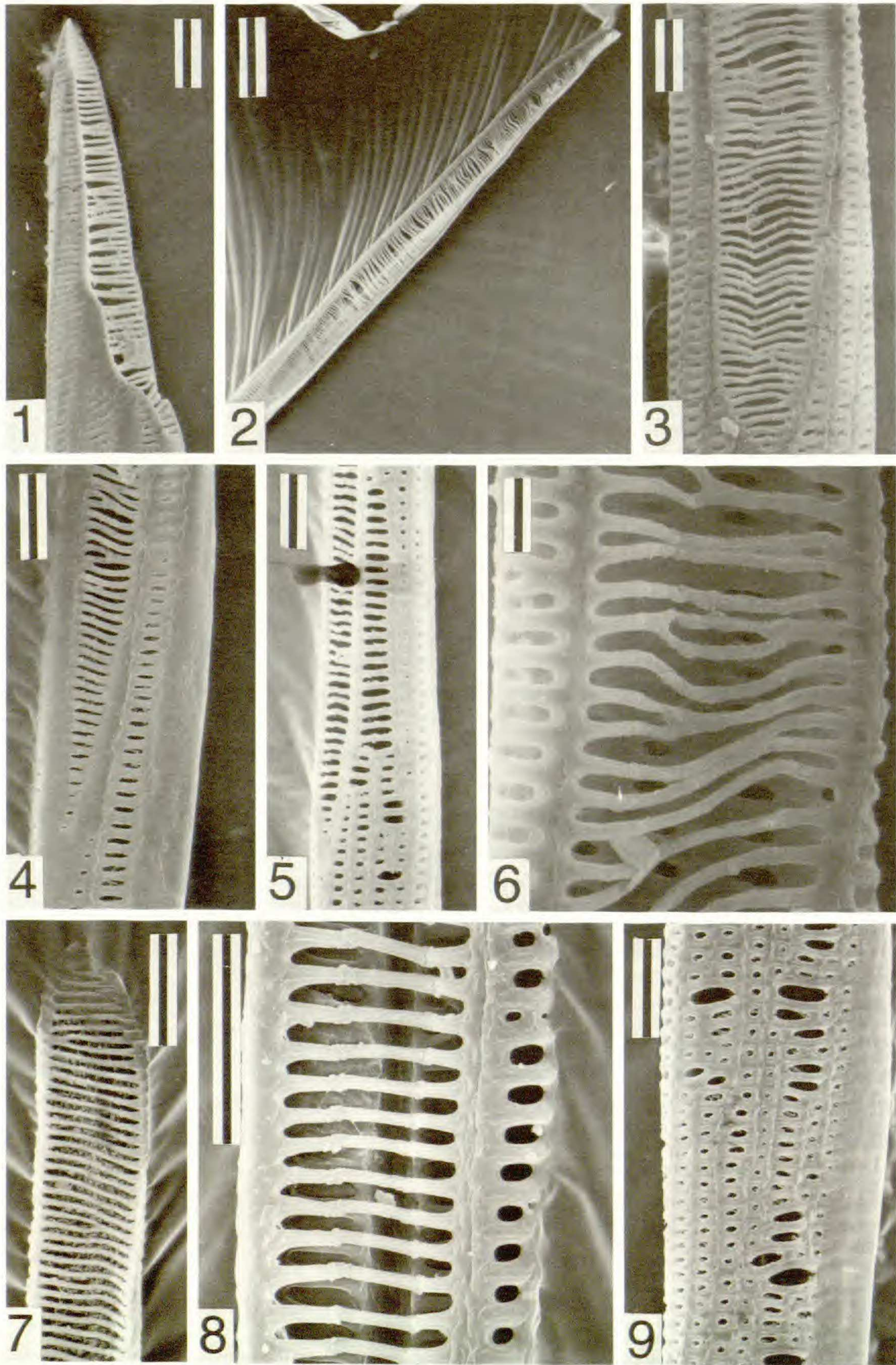
We have observed features of vessel elements in roots and rhizomes from *Matteuccia struthiopteris* and *Osmunda cinnamomea* var.*asiatica* and found that even if they belong to the same ecological grouping, their vessel elements show different patterns of specialization, i.e. primitive *O. cinnamomea* var. *asiatica* had little differentiation between perforation plate on the end wall and lateral wall pitting whilst *M.struthiopteris* was markedly differentiated (Li et al., 1999). Thus we choose those different taxa, which are in different systematic positions, in the same ecological group as research materials to observe the microstructures of tracheary elements by means of SEM for understanding evolutionary trends of tracheary elements of ferns in similar and/or the same environment.

Dryopteris Adans. (Dryopteridaceae) has about 400 species distributed mainly in the North Temperate Zone (Wu & Ching, 1991). Russow (1873) considered that roots of *Nephrodium* (= *Dryopteris*) had true vessels because of the combination of lateral pitting which was most often alternate or opposite with short scalariformly pitted overlapping areas between the tracheary elements. Schneider and Carlquist (1997) have documented presence of vessels in *Polystichum* of the same family by means of SEM.

Dryopteris crassirhizoma Nakai is common in Heilongjiang province, which belongs to the second ecological species group but it also intrudes into other habitats including dry places. We attempt to demonstrate with SEM whether tracheary elements in root and rhizomes of this species are vessels or tracheids and make a comparison of microstructures of tracheary elements to these of *Adiantum pedatum* in the same ecological group and *Polystichum acrostichoides* in the same family.

MATERIALS AND METHODS

Fresh roots and rhizomes of *Dryopteris crassirhizoma* were collected from 3–5 wild plants of the Maoershan population, Shangzhi County, Heilongjiang province, and fixed in 3:1 absolute ethanol-glacial acid for 2–24 hours. Fixed materials were transferred into 70% aqueous alcohol and stored. We used mac-



FIGS. 1–9. SEM photographs from tracheary elements in root of *Dryopteris crassirhizoma*. 1. Lateral view of relative short end wall. 2. Face view of elongate end wall, scale bar = 50 μm. 3. The lower part of the end wall, to show wider scalariform perforations in end wall that contrast with

erated and sectioned materials for SEM observation. We use two maceration methods (Franklin's method: solution consisted of glacial acetic acid and 3% hydrogen peroxide (1:1) and Jeffrey's method used by Carlquist et al. 1997). Roots and rhizomes were macerated in Franklin solution at 60°C for 4–5 days, while in Jeffrey solution at 23°C for 2–3 days. These macerated materials were transferred into 70% alcohol, then spread onto the surface of SEM aluminum stubs. After drying, they were sputtercoated and examined with a Hitachi S-520 SEM. The sectioned materials were made as follows: the materials fixed in FAA were cut longitudinally, and dehydrated by an ethanol series. Then the ethanol was removed by isoamylacetate. The materials were dried in a critical point dryer with carbon dioxide and coated with gold.

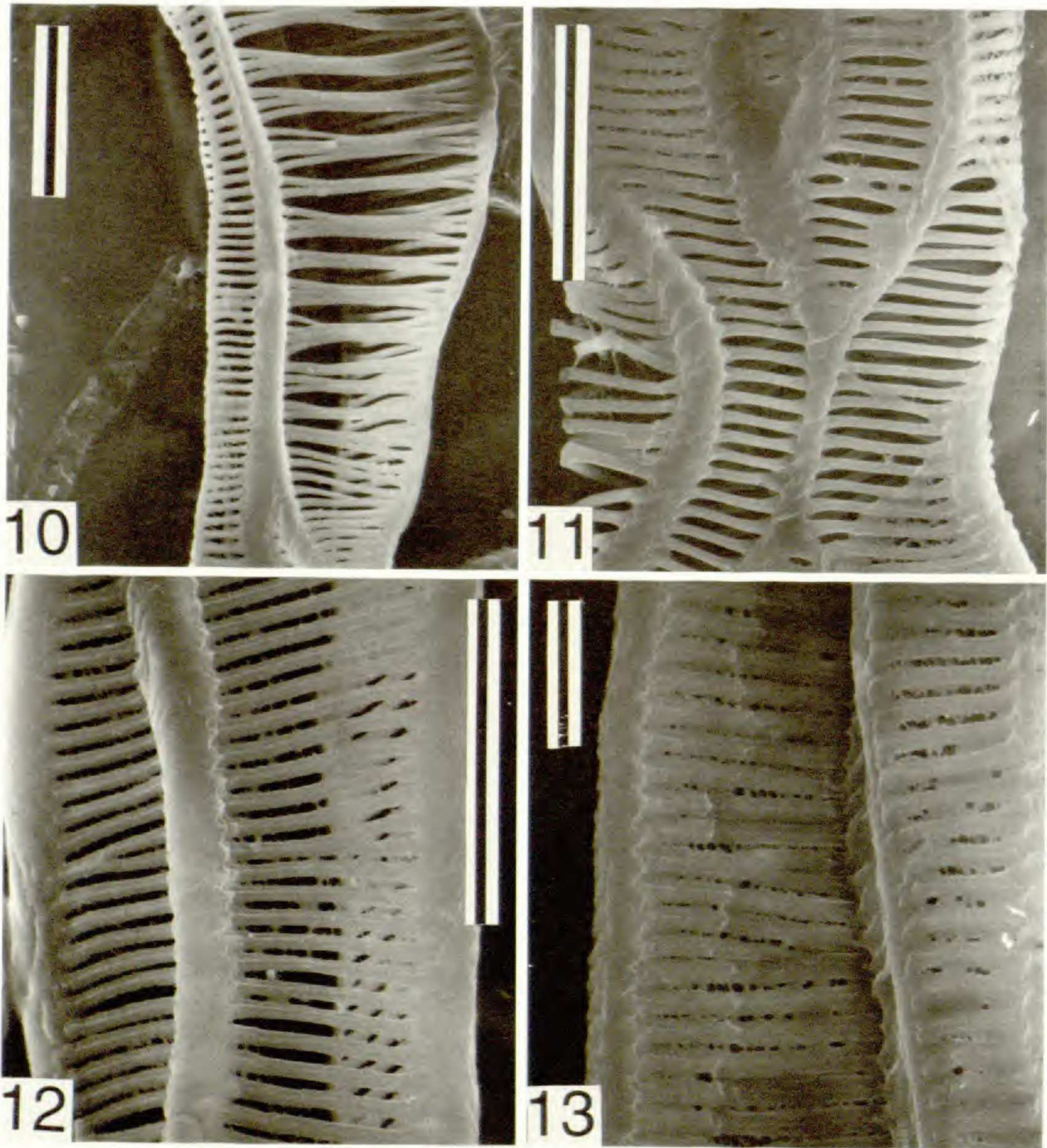
RESULTS

In *Dryopteris crassirhizoma* roots, all end walls of tracheary elements examined bear perforations. These perforation plates range widely in morphology. Some perforation plates are relatively short with numerous bars (Fig. 1), whereas other perforation plates are very long with numerous bars (Fig. 2). Perforation plates are markedly differentiated from lateral walls; the perforations on end walls are larger and more elongate than lateral-wall pits or perforations arranged in alternate or opposite pattern (Figs. 3–5). In figure 5, the end wall is at the left, but some lateral-wall perforations are similar to the end-wall perforations in absence of pit membrane remnants. In some plates, pit membrane remnants are very few (Fig. 6), but sometimes relatively abundant (Fig. 7). In the end wall shown in figure 8, monomorphic perforations mostly lack pit membranes. Some end walls show dimorphism between wide and narrow perforations in figure 6. In addition to perforation plates on end walls, lateral-wall perforations are also found (Figs. 5,8,9). It is interesting that lateral-wall perforations range from very small to large; several larger perforations (usually two to four perforations) usually group together (Fig. 9).

Most tracheary elements in rhizomes possess end walls with perforations, which are less specialized than those in roots (Fig. 10). Some wider perforations on end walls clearly lack pit membranes in this figure and those narrow perforations that alternate with wide perforations in the same perforation plate usually have various degrees of pit membrane remnants (i.e. from intact pit

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the alternate or opposite pits or narrower perforations with pit membrane remnants in lateral wall. 4. The lower part of end wall, to show those perforations with absence of pit membrane. 5. The lower part of end wall, to show those perforations with absence of pit membranes and lateral-wall perforations in the right of end-wall perforation plate. 6. End wall, to show perforations with few pit membrane remnants and dimorphism in wideness of perforations; grain of unknown materials lodged on perforation plate. 7. End wall, to show perforations with many pit membrane remnants. 8. Central portion of an end-wall perforation plate, to show monomorphic perforations that completely lack pit membrane. 9. Lateral-wall perforation plate, to show larger perforations grouping in lateral wall. Scale bars = 30µm.



FIGS. 10–13. SEM photographs from tracheary elements in rhizomes of *Dryopteris crassirhizoma*. 10. Perforations with absence of pit membrane and the lower perforations with some pit membrane remnants on end wall; 11. Central section of this vessel, to show four perforation plates. 12. Lateral-wall perforations with porose pit membranes of vessel element shown in figure 10. 13. Lateral wall, to show various sizes of pores in pit membrane, scale bar = $10\mu\text{m}$. Scale bars in the other figures = $30\mu\text{m}$.

membrane to nearly devoid of pit membrane remnants). The majority of lateral walls consist of scalariform perforations in which there are porose pit membranes or pit membrane remnants (Figs. 12, 13), but in some lateral walls, perforations markedly lack pit membranes as is the case for end-wall perforations. One can find many tracheary elements that have several facets in contact with other tracheary elements. The tracheary elements possess as many as eight facets with long, scalariform pits, which have entire pit membranes, porose pit membranes, or degrees of pit membrane remnants (Fig. 11).

DISCUSSION

In the present study, *Dryopteris crassirhizoma* has been shown to possess vessels in both roots and rhizomes, and the same characters of these vessel elements in materials made by three methods are observed by mean of SEM. Our SEM observations not only reveal that pits on oblique end walls as seen in the light microscopy are perforations which lack pit membranes, we also find perforations with degrees of pit membrane remnants on lateral walls. *Dryopteris crassirhizoma* therefore has end-wall perforation plates and lateral-perforation plates. End-wall perforation plates in roots are more specialized than in rhizomes; these perforation plates are obliquely positioned in end walls. End walls in roots are relatively short with wider scalariform perforations that contrasts with the alternate or opposite pits or narrow perforations with pit membrane remnants on lateral walls. Thus end walls are markedly differentiated from the lateral walls. Moreover their perforation plates are more specialized than those of *Adiantum pedatum* belonged into the same ecological group (Li et al., 1999) and *Polystichum acrostichoides* in the same family (Schneider and Carlquist, 1997) based on morphology of perforation plate on the end wall. *Dryopteris crassirhizoma* not only occurs in mesic habitat, but also ranges into other habitats such as dry place and grow normally. In the genus *Woodsia*, *W. scopulina* and *W. ilvensis*, which occurs in places where winter freezing and summer drought abbreviate the growing season, all had high differentiation in morphology between perforation plate and lateral wall pitting, in contrast, *W. obtusa* growing in a mesic habitat had little differentiation (Carlquist, Schneider and Yatskievych, 1997; Schneider and Carlquist, 1998a; Carlquist and Schneider, 1998a). This phenomenon was explicated to markedly differentiated vessels as a benefit to increase water supply when ferns occupied area where there was water availability stress in some seasons.

Carlquist and his colleagues observed transverse perforation plates with several bars in vessels of *Pteridium* roots (Carlquist and Schneider, 1997a) and no lateral-wall perforation plates in *Woodsia obtusa* (Carlquist, Schneider and Yatskievych, 1997). It is evident that vessels of *Dryopteris crassirhizoma* are moderately specialized. In rhizome vessels, narrow scalariform perforations of end walls are in contrast to the scalariform pits and perforations with pit membrane remnants on the lateral walls; there is less differentiation between end walls and lateral walls. This result is observed in several other ferns (Carlquist & Schneider, 1997a, b; Carlquist et al., 1997; Schneider & Carlquist, 1997), which supplies evidence that vessels may have first occurred in fern roots. Dimorphic perforations are common in vessels of roots and rhizomes; Figure 9 shows the end walls of some root vessels which have perforations differentiated into two forms. This dimorphism in width of perforations also occurs in end walls of rhizome vessels, but it is less pronounced.

Lateral-wall perforations are common in *Dryopteris crassirhizoma*. We noted some particularly larger perforations which differ markedly from the other perforations in the same lateral wall shown in figs. 5, 8 and 9, which (two to four) group together to form a local perforation district or plate. In *Astropis*,

Phlebodium, *Polystichum*, *Pteridium* and *Woodsia*, this phenomenon is newly reported. Gwynne-Vaughan (1908) considered that the formation of lateral-wall perforations in *Pteridium* was related to abundant tyloses, but further study is needed on the development of this type of perforations in lateral walls of *Dryopteris crassirhizoma*.

The lateral walls of rhizome vessels have porose pit membranes. Perforations of the terminal end wall of vessels in roots and rhizomes may also have this type of membrane. Carlquist and Schneider (1997a) considered that presence of porose pit membrane in *Pteridium* vessels could enhance lateral transport of water without markedly increasing the vulnerability of vessels to transfer of air embolism from one vessel element to another laterally. End walls are less specialized, because numerous pits with porose pit membranes and/or pit membrane remnants occur in lateral wall of rhizomes. We note that some vessels in rhizomes have several facets, in which long scalariform pits have various degrees of porose pit membrane, ranging from smaller pores in pit membranes to absolute absence of pit membranes. These vessel elements contact with several other cells for transferring materials.

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